

EUPLOTES RARISETA SP. N. (PROTOZOA : CILIATEA) A NEW SMALL MARINE HYPOTRICH

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INTRODUCTION

TUFFRAU (1960) listed 55 specimens of the genus *Euplotes* Ehrenberg, 1830 that had been described to that date, and in his extensive revision of the genus eliminated many of the species. In doing so, Tuffrau (1960) suggested that there were four characters of fundamental importance which should be used for taxonomic purposes at the species level. These characters were: the number of laterodorsal kineties, the arrangement of the dorsal silver-line network, the number of frontoventral cirri and the shape of the non-dividing macronucleus. Borror (1968) added the appearance of the pellicular or cortical sculpturing that is often a feature of some *Euplotes* spp. as a further criterion. Using these characters, in addition to some of the more traditional features, Borror (1972) listed 43 species of *Euplotes* in his revision of the order *Hypotrichida* Stein, 1859.

When both traditional and modern criteria are considered, the species of *Euplotes* described in the present paper does not conform to any of those listed by Borror (1972) nor Carter (1972).

MATERIALS AND METHODS

(a) *Source and cultivation*

The hypotrich was originally contained in a sample of seawater collected from Aberystwyth, Wales. Some seawater was inoculated into a flask of Erdschreiber solution ('Medium 1', Committee on Cultures, Society of Protozoologists, 1958) which was then incubated in the dark at room temperature (about 20 °C) for several weeks. Under these conditions, *Euplotes* was the dominant ciliate which grew. Some of the cells so obtained were used by Miss Sheila Andrews to initiate a culture at the Cambridge Culture Centre of Algae and Protozoa (*Euplotes* sp. LB 1624/2a). Later, a clonal culture was established (*Euplotes* sp. LB 1624/2b) and most of the work described herein relates to that clone.

The hypotrich was maintained in the dark at room temperature in conical flasks or test tubes containing Erdschreiber solution. Cultures were fed weekly with a few drops of a thick suspension of freeze-dried baker's yeast and subcultured at monthly intervals. The ciliate also grew well in 150 ml conical flasks containing 100 ml of seawater and 10–12 rice grains, but the maximum populations reached in these cultures were lower than those achieved by the former culture method. In order to establish the possible ecological range of this species, some experimental work was carried out with media consisting of various dilutions of Erdschreiber solution supplemented with baker's yeast and seawater supplemented with rice grains. In these

experiments, duplicate cultures in each medium were subcultured serially into flasks containing media of progressively lower salinity at 10 per cent (v/v) intervals. The inoculum size was always 4 ml per 100 ml of medium and a period of 1–5 weeks at room temperature was allowed between each transfer. In this way it was possible to establish the approximate lower salinity limit for this species.

(b) *Light microscopy*

Observations and measurements were made using both living and fixed material. Living organisms were slowed by immersion in methyl cellulose (5 per cent w/v in seawater), and osmium tetroxide vapour (2 per cent w/v) was found to be a suitable fixative for cells which were to be drawn or measured. Large numbers of *Euplotes* were fixed in bulk and their nuclei were stained using Dippell and Chao's modification of De Lamater's basic fuchsin stain (Sonneborn, 1950). The silver-line system was displayed using the 'wet' method modified by Chatton & Lwoff (1930) and Corliss (1953).

(c) *Scanning-electron microscopy*

The preparation of these marine organisms for the scanning-electron microscope proved to be rather more complex than the method described by Small and Marszalek (1969). It was found necessary to wash the cells 5 or 6 times prior to fixation in order to separate the cells from bacteria, yeast and debris. The first two washes were carried out by slow centrifugation (approx. 400 g) for a few seconds. In this case the majority of the *Euplotes* were retained in the supernatant. The cells were then repeatedly washed in membrane-filtered seawater and centrifuged at approximately 1750 g. The cells in the final pellet were rapidly killed in osmium tetroxide vapour to prevent the cirri curling during fixation. Considerable effort was made to find a good method of fixation for these marine ciliates which was suitable for scanning-electron microscopy. The fixative suggested by Small & Marszalek (1969) was that used by Parducz (1967) and although this was acceptable in some respects, it had the disadvantage of disrupting the structure of the cirri and the adoral zone of membranelles (AZM). The fixative finally chosen, although it is still under review, consisted of equal parts of 2 per cent (w/v in distilled water) osmium tetroxide and a saturated aqueous solution of mercuric chloride. Sufficient sodium chloride was then added to give a final concentration of 3.3 per cent (w/v); this prevented the cells swelling during fixation. The three component parts were mixed immediately before use and then membrane filtered; cells were fixed in the filtered solution overnight. Following fixation, the cells were washed repeatedly (at least 8 times) in membrane-filtered triple glass-distilled water and centrifuged at approximately 400 g between each wash. The washed cells were then frozen by being dropped onto pieces of aluminium floating on liquid nitrogen and were dried in a tissue dryer essentially as described by Small & Marszalek (1969). The pieces of aluminium covered with organisms were glued to stubs and coated with approximately 100 Å of gold as described by Harris, Martin & Ogden (1972), specimens were examined with a Cambridge 'Stereoscan' Mk II scanning-electron microscope (Cambridge Instruments Ltd, Cambridge, England).

Euplotes rariseta sp. n.*Diagnosis*

Small (30–45 μm long, 20–31 μm wide), ovoid, marine hypotrich with 10 fronto-ventral, 5 transversal and 3 caudal cirri; caudal cirrus below AZM stout. Ventral surface heavily sculptured with 6 posteriorly projecting ridges. Dorsal surface with 6 double-edged longitudinal ridges. Dorsal bristles sparse; 6 kineties with a maximum of 6 bristles in the central kineties. Dorsal silver-line system double of the *patella* type. Macronucleus S-shaped. Micronucleus small, situated anteriorly.

Type slides showing silver-line systems, nuclear apparatus and whole mounts have been deposited in the slide collection of the Protozoa Section, BM (NH): holotype Reg. No. 1972:11:1:11; paratype Reg. Nos. 1972:11:1:12–18.

Body size and shape

The size distribution data of *Euplotes* cells at the stationary and logarithmic phases of growth are given in Fig. 1. It should be noted that *Euplotes* cells from the logarithmic phase are considerably larger ($42 \pm 3 \mu\text{m}$ long by $28 \pm 3 \mu\text{m}$ wide) than are cells from the stationary phase ($34.6 \pm 3 \mu\text{m}$ long by $24.2 \pm 2.3 \mu\text{m}$ wide). This is in agreement with the observations of Curds & Cockburn (1971) who noted that the size of the ciliate *Tetrahymena pyriformis* was related to its rate of growth. The data on size show that this species of *Euplotes* is amongst the smallest recorded for the genus.

The dorsal surface is conspicuously sculptured with 6 double-edged longitudinal ridges (Pl. 1, figs. a and c). To the left of each ridge is a parallel row of pits from which the short (2 μm long) dorsal cilia or bristles emerge. The ventral surface is also strongly sculptured (Fig. 2; Pl. 1, figs. a and c), particularly in the posterior region between the transverse cirri. There are 6 ventral ridges, 3 of which are prominent and consist of an oblique midventral ridge, and 2 ridges which run longitudinally flanking the 2 outermost transverse cirri. The other 3 ridges are less prominent and are more or less restricted to the area immediately between the transverse cirri. All 6 ventral ridges project slightly posteriorly between the transverse cirri and form fin-like structures (Fig. 2; Plate 1, figs. b and c). The right buccal overture has a slight anterior evagination as in *Euplotes alatus* Kahl, 1932 (see Borror, 1968) but in the species under description it extends only just past the AZM.

Ciliary organelles

This *Euplotes* has 10 frontoventral cirri which are arranged as shown in Pl. 1, fig. b and in Figs. 2 and 3b. The arrangement closely resembles that of both *Euplotes charon* Ehrenberg, 1830 and *Euplotes quinquecarinatus* Gelei, 1950 (see Borror, 1968). There is the normal complement of 5 transverse cirri which arise between the ventral ridges and these, particularly cirri III 1 and IV 1 (following the numeration of Wallengren, 1900), are frequently seen to be frayed at the distal end (Fig. 2). The tendency for the transverse cirri to disrupt in this way is greatly increased during fixation. There are only 3 caudal cirri, and in this species the one

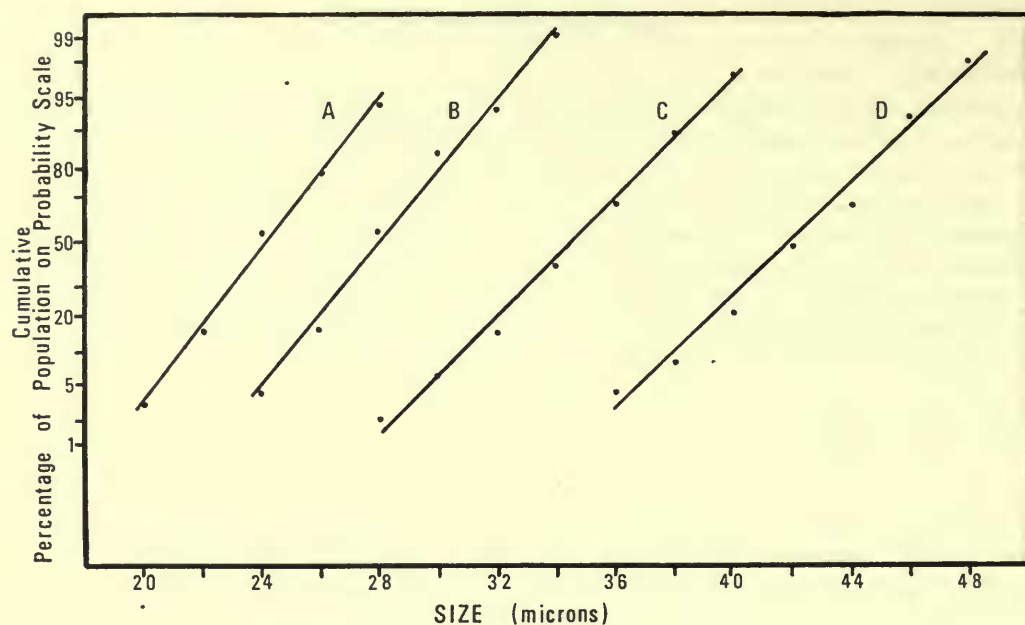


FIG. 1. Size distribution data of *Euplotes variseta*. Curve A. Breadth of cells at stationary phase. Curve B. Breadth of cells during logarithmic growth. Curve C. Length of cells at stationary phase. Curve D. Length of cells during logarithmic growth. Stationary-phase cells were fixed in osmic acid vapour. Logarithmic-phase cells were measured alive immersed in methyl cellulose solution.

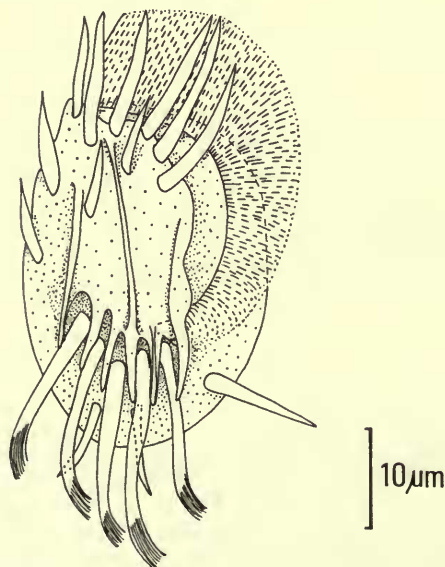


FIG. 2. Ventral aspect of *Euplotes variseta* showing cirri and ventral ridges (camera lucida drawing).

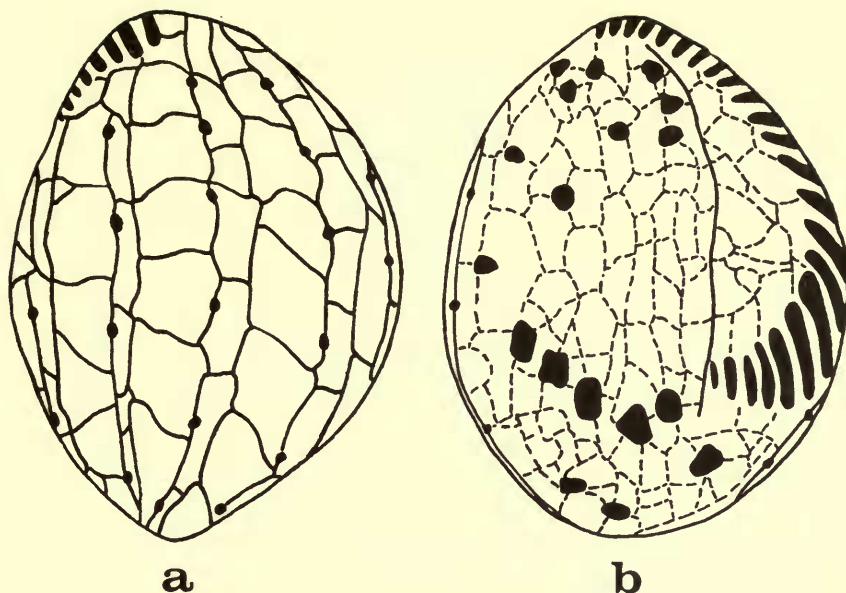


FIG. 3. Silver-line system of *Euplotes rariseta*, (a) dorsal surface, (b) ventral surface.

below and nearest to the AZM is characteristically held stiffly out to the left side and is much stouter than the other two caudal cirri which arise beneath the central transverse cirri (Fig. 2). The presence of this large stiff caudal cirrus appears to be a feature unique to this species; in the living cell, the cirrus seems to act as a rudder-like structure. The dorsal bristles are sparse in this species; there are 6 rows and the mid-dorsal rows have only 6 bristles, in which respect this species resembles *Euplotes bisulcatus* Kahl, 1932 (see Borror, 1968).

Nuclei

The macronucleus of this species is an irregular S-shape (Fig. 4). In this respect it appears to be unique in the genus *Euplotes*. The posterior part of the macronucleus points anteriorly and ventrally towards the position of the cytostome. The micronucleus is situated close to the macronucleus on the left anterior edge of the body (Fig. 4).

Silver-line system

The geometry of the dorsal silver-line system is of the double or *patella*-type and closely resembles that of *Euplotes raikovi* Agamaliev, 1966 with a series of alternate longitudinal rows of narrow long polygons and wide short polygons (Fig. 3a). The ventral argyrome (Fig. 3b) consists of an irregular network of polygons in similar numbers to that of *E. cristatus* Kahl, 1932 (see Tuffrau, 1960). The dorsal silver-line network has been seen on some scanning-electron micrographs (Pl. 1, fig. d).

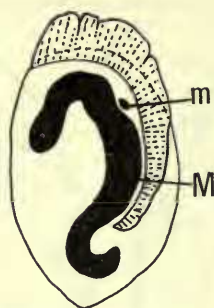


FIG. 4. Ventral view of the nuclei of *Euplotes variseti* from a stained preparation. (M = macronucleus, m = micronucleus.)

Ecological data

Cells inoculated into Erdschreiber solution supplemented with yeast grew well in nine different dilutions of this medium from 100 down to 20 per cent (v/v). Cells transferred from 20 to 10 per cent Erdschreiber solution showed no growth during the 6 weeks following inoculation. Growth in seawater supplemented with rice grains was good in seven dilutions from 100 down to 40 per cent (v/v), but cells transferred from 40 to 30 per cent seawater showed good growth only after 3 weeks in culture. Subsequent transfer of cells from 30 to 20 per cent seawater led to poor growth after 5 weeks, and no growth was observed in 10 per cent seawater. Contractile vacuole activity was not observed in organisms from any of the tested dilutions of seawater or Erdschreiber solution. This is in agreement with the findings of Yocum (1934), who found that the activity of the contractile vacuole in *Euplotes patella* Ehrenberg, 1831 diminished and became imperceptible at concentrations of seawater above 10 per cent. Yocum (1934) also found that *E. patella* would survive well and divide in 66 per cent seawater. In view of the finding that the species of *Euplotes* described in the present paper will survive and divide in dilutions of seawater or Erdschreiber solution between 20 and 100 per cent, it seems likely that, in nature, this organism could be found in both marine and brackish environments. However, for the lower dilutions tested, organisms were not as abundant in seawater medium as they were in Erdschreiber medium, and it is possible that in these experiments the amounts of substances other than sodium chloride were limiting growth.

DISCUSSION AND CONCLUSIONS

In the past, some authors have noted that the number of caudal cirri is not as constant as the others, for example, *Euplotes mutabilis* Tuffrau, 1960 has 4–5 caudals, *E. raikovi* has 2–3 caudals and there are other similar examples. In view of these observations perhaps the use of the number of caudal cirri as a character should be regarded with caution. Although *E. octocirratu*s Agamaliyev, 1967 is the only other species of *Euplotes* which has precisely the same numbers of cirri on the ventral surface, that is 10 frontoventrals, 5 transversals and 3 caudals, one should

consider those species with a 10 : 5 : 4 cirri complement. These include the following : *E. alatus*, *E. balteatus* Kahl, 1932, *E. crenosus* Tuffrau, 1960, *E. cristatus*, *E. harpa* Stein, 1859, *E. indentatus* Carter, 1972, *E. inkystans* Chatton in Tuffrau, 1960, *E. minuta* Yocum, 1930, *E. magnicirratu*s Carter, 1972, *E. quinquecarinatus*, *E. roscoffensis* Dragesco, 1966, *E. trisulcatus* Kahl, 1932, *E. tuffraui* Berger, 1965 and *E. vannus* Minkjewicz, 1901. In all these species the shapes of the macronuclei are quite unlike that of the species described in this paper and furthermore the silver-line systems of these species are different.

It is unfortunate that the dorsal and ventral silver-line systems have not been described for all the known species of *Euplotes* ; however, of those listed above none have 6 dorsal kineties as found in *E. rariset*a ; only *E. octocirratu*s and *E. trisulcatus* have 7 kineties, the remainder have 8 or more. There are four other species with 6 dorsal kineties and these are *E. raikovi*, *E. strelkovi* Agamaliyev, 1967, *E. tegulatus* Tuffrau, 1960 and *E. balticu*s Kahl, 1932. None of these four species have the correct numbers of cirri and furthermore all possess far too many dorsal kinetosomes. *E. bisulcatus* is the only other species with a maximum of 6 dorsal kinetosomes per kinety but there are 8 dorsal kineties in that species.

*Euplotes rariset*a differs from all previously described species of *Euplotes* in possessing an S-shaped macronucleus and a very stout caudal cirrus. In addition, although the remaining characters have been observed amongst other species of *Euplotes*, it is evident from the information given above that none of these species has the same combination of characters. For these reasons, it is considered that the species of *Euplotes* described in this paper is sufficiently distinct from all others to be designated as a separate species, and because of the paucity of dorsal bristles has been named *Euplotes rariset*a. Following the revised classification of the Committee on Taxonomic Problems of the Society of Protozoology (Honigberg *et al.* 1964) *Euplotes rariset*a is placed into class Ciliata Perty, 1852, order Hypotrichida Stein, 1859, family Euplotidae Ehrenberg, 1838.

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PLATE 1

Scanning micrographs of *Euplotes variseta*

- a. Dorsal view showing ridges and rows of dorsal bristles ($\times 2.1k$)
- b. Ventral view showing the arrangement of cirri and ventral ridges ($\times 1.8k$)
- c. Posterolateral aspect showing double-edged dorsal ridges ($\times 2.7k$)
- d. Dorsal surface showing argyrome ($\times 6.1k$)

